Tribioscience

gamma-Glutamyl Transferase, Enzyme Activity

Catalog	Unit
TBP0068-100U	100 U
TBP0068-500U	500 U

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 20-50 U/mg

Protein: 90%

Unit Definition

That amount of enzyme causing the release of one micromole of p-nitroaniline per minute at 37°C and pH 8.2.

Assay Method

The method of assay is based on that of Szasz in Methods of Enzymatic Analysis, in which the rate of increase in absorbance due to release of p-nitroaniline is measured at 405 nm and 37°C.

Applications

((5-Glutamyl)-peptide:amino acid 5-glutamyl transferase; EC 2.3.2.2) Gamma glutamyl transferase (g-GT) catalyzes the following reaction:

g-GT 5-Glutamyl-p-nitroanilide + Glyclglycine ------> 5-Glutamyl-glycylglcine + p-Nitroaniline

Gamma glutamyl transferase is found in the microvilli of the small intestine and in the kidney brush border plasma membrane. It is an important liver enzyme and high serum levels are associated with liver disorders.

Reagents

1. 0.16 M Glycylglycine. Dissolve 20.5 mg/ml in 0.05 M Tris (free base).
2. 0.016 M Magnesium chloride●6H2O. Dissolve 3.3 mg/ml in 0.05 M Tris (free base).
3. 0.05 M Tris (free base).
4. 120 mg Gamma-glutamyl-p-nitroanilide.
5. Enzyme solution (0.1-0.2 U/ml). Dissolve enzyme in ice-cold 0.05 M Tris-HCl buffer, pH 8.2 immediately prior to assay.

Procedure

1. Set spectrophotometer (equipped with strip chart recorder and temperature control) at 405 nm and 37°C.
2. Into a beaker mix the following reagents. Stir at 50°C until dissolved and adjust the pH to 8.2 with 1 M HCl or 2 M NaOH.

Glycylglycine	33 ml
Magnesium chloride	33 ml
Tris	33 ml
Gamma-glutamyl-p-nitroanilide	120 mg

3. Pipette 2.90 ml of the substrate solution into a cuvette and incubate in the spectrophotometer at 37°C for 10 minutes to attain temperature equilibration.
4. Record blank rate at 405 nm.
5. Add 0.10 ml of the enzyme solution to the cuvette. Mix and record the increase in absorbance at 405 nm for 5-8 min.
6. Calculate the E405 nm/min) from the linear portion of the curve.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{405nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(9.9)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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